THE EFFECT OF ULTRA-VIOLET RADIATION ON MICROÖRGANISMS WITH SPECIAL EMPHASIS ON ASHBYA GOSSYPII AND ITS SYNTHESIS OF RIBOFLAVIN

BY

THOMAS GRENVILLE PRIDHAM

B.S., University of Illinois, 1943
M.S., University of Illinois, 1946

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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THOMAS GRENVILLE PRIDHAM

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The principal objective in this study was to develop substrains of *Ashbya gossypii* characterized by increased synthesis of riboflavin (Vitamin B_2, Vitamin G). This goal has been realized. Several substrains have been isolated which produce yields of riboflavin 10 to 20 per cent higher than the parent culture. At the same time, numerous mutant strains with degenerate cultural and structural characteristics and reduced riboflavin synthesizing capacities have been isolated and examined. Such improved and degenerate substrains originated both through natural variation and ultra-violet induced mutation of the parent culture NRRL Y-1056. The results are consistent with those reported by other investigators in work of a similar nature with other microörganisms. As a corollary to this study, the laboratory-scale flask fermentation procedure has been improved to a point where yields of 800 to 1000 micrograms of riboflavin per milliliter of unfiltered fermented substrate are consistently obtained.

A comprehensive survey of the literature has shown that of the many microörganisms tested for ability to synthesize riboflavin, only representatives of seven genera, i.e., *Clostridium, Mycobacterium, Candida, Saccharomyces, Torulopsis, Eremothecium,* and *Ashbya,* produce the compound in sufficient quantity to warrant industrial exploitation. Of these, the latter two are in a class by themselves because of the exceptionally high flavinogenic capacities of certain species and strains. Employing selected strains of *Eremothecium ashbyii* or *Ashbya gossypii,* it is not unusual to realize yields of 500 to 1000 micrograms of riboflavin per milliliter of fermented mash and up to 50,000 micrograms per gram of dried fermented residue. Yields by other micro-

organisms do not begin to approach these figures.

Aside from the ability of certain strains of *A. gossypii* (Ashby and Nowell) Guilliermond to synthesize prodigious amounts of riboflavin, the organism is of further interest as an important plant pathogen and as a species which exhibits a specific nutritional requirement for biotin. While considered as a yeast-like fungus in the order Endomycetales by most investigators, relationships to other microörganisms are still not clear and pose an interesting taxonomic problem.

The use of ultra-violet radiation was suggested by a thorough survey of the literature concerning the effects of ultra-violet light on microörganisms. Such treatment has been demonstrated to induce cultural, morphological, and metabolic changes in microörganisms, including the occasional capacity for increased synthesis of compounds of industrial importance. In the current investigation, particular emphasis was placed upon the isolation of strains showing greater capacities for riboflavin synthesis. Concurrent morphological, cultural, and physiological studies were carried out with each strain isolated.

Little evidence of natural variation was obtained in this study. Nevertheless, some colonies showed a tendency to form sectors of less intense pigment. This indicates an inherent tendency for the parent to revert to a less flavinogenic form. There was also some evidence of mutation in the opposite direction since several substrains produced more riboflavin than the parent. NRRL Y-1056, the strain upon which the current work is centered, is of such origin.

A few strains originating from spores subjected to ultra-violet radiation at 2537 Angstrom units for varying lengths of time up to 15 minutes have been demonstrated to synthesize 10 to 20 per cent more riboflavin than the parent strain. The increased flavinogenic capacities of the irradiated substrains were shown to be heritable in several instances.

The majority of the strains studied produced amounts of riboflavin either equivalent to- or less than- those of the

parent, a phenomenon generally encountered among artifically induced mutants. To a certain extent, the possession of obviously altered morphological, cultural, and physiological characteristics could be correlated with subsequent low flavinogenic abilities. Substrains producing increased yields were culturally and morphologically indistinguishable from the parent.

A considerable number of ultra-violet treated strains were obtained which evidenced altered characteristics of a degenerate nature. These included strains characterized by thin hyphae, or aberrant branching; strains in which spore and sporiferous sac formation was depressed or completely inhibited; strains characterized by aberrant sporiferous sacs; strains forming colonies of a more compact nature or with more ragged advancing edges than those of the parent; strains showing considerable tendency to form sectors of less intense pigment; and strains in which pigment formation was depressed or completely inhibited. Two physiological mutants were obtained which were capable of synthesizing considerable amounts of riboflavin in a completely synthetic medium in which the parent produced no visible pigment. Several other physiological mutants unable to grow on 'minimal' media were obtained but not further studied.

Retesting of a number of these isolates showed the majority of altered characteristics to be non-heritable, probably representing temporary effects of the radiation on the spores originally exposed. a few were shown to be heritable and might represent true mutations, or possibly 'Dauermodifikationen.' A number of the strains possessed more than one altered characteristic.

Consistently high yields of the vitamin were obtained with the parent strain when cultivated in a medium composed of corn steep liquor 2.5 per cent, peptone 0.5 per cent, and commercial glucose 4.0 per cent, provided inocula were prepared and employed properly. It was shown that extremely small amounts of inoculum of the order of ¼ per cent by volume led

to the highest yields. Investigation of other factors necessary for optimal production of riboflavin indicated that phosphates were stimulatory, while small amounts of most other inorganic salts were either inhibitory or had no effect.

It is concluded that ultra-violet radiation has the capacity for inducing changes of a degenerative nature in *Ashbya gossypii* NRRL Y-1056 correlated with decreasing flavinogenic ability. To a lesser degree it has the capacity for inducing variations characterized by higher riboflavin synthesizing capacities. Natural variation occurs to only a slight extent and may lead either toward or away from prodigious flavinogenic ability. Furthermore, in the organism, the mechanisms for exceptional synthesis of riboflavin are delicately balanced, and respond markedly to variations in the composition of the substrate and to variations in the procedures employed in carrying out the fermentation.

VITA

Thomas Grenville Pridham was born October 10, 1920 in Chicago, Illinois. He attended elementary schools in Moline, Illinois, and was graduated from Moline High School in 1938 with Honors in Chemistry. In the same year he began college work at the University of Illinois aided by a LaVerne Noyes Scholarship which he held for two years. He was graduated with the B.S. Degree in Chemistry in 1943.

During the course of his undergraduate work he was employed as a laboratory assistant in the Botany Section of the Illinois State Natural History Survey, Urbana, Illinois, and in the Department of Animal Husbandry, University of Illinois. He was employed for a brief period as a War Department Inspector of Powder and Explosives in the Iowa Ordnance Plant, Burlington, Iowa.

He was called into active duty with the United States Naval Reserve in March 1943 and served as a communications officer aboard an LST in the Central Pacific area. After release to inactive duty he resumed his university work, being aided by half- and full-time teaching assistantships in the Department of Bacteriology, University of Illinois. He was awarded the M.S. Degree in Bacteriology in 1946. In 1948 he joined the staff of the Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Peoria, Illinois where he completed his thesis work for the degree of Doctor of Philosophy in Bacteriology.

The author is a member of Sigma Xi, The American Association for the Advancement of Science, The Society of American Bacteriologists, The Society of Illinois Bacteriologists, and the American Chemical Society.

In June, 1948, he was one of 100 naval officers

chosen from throughout the United States to participate in the first Scientific Seminar sponsored by the Office of Naval Research in Washington, D. C.

He is married and has two children.

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